Suppl. Table 1

Supplementary Table 1. Clinical characteristics of LUD00-0018 cohort

		Diagnostic				Disease at Study entry			Vaccine Protocol			Outcome			
Vaccine	Patient	Sex	Age	TNM	Breslow	Stage	Status	Previous treatments	Protocol	Duration†	# of vaccine	Relapse	Death	PFS*	OS*
ELA	LAU 205	M	24	pT2aN1bM0	1.40	III	NED	Surgery, immunotherapy (c)	Group IV (ELA+TYR)	25.0	20	yes	yes	25.0	50.0
	LAU 321	M	60	pT3aN0M0	1.50	IV	ED	Surgery, chemotherapy, limb perfusion, immunotherapy (a)	Group I (ELA)	7.0	8	yes		2.1	75.0
	LAU 371	M	29	pT3aN1aM0	2.38	IV	NED	Surgery, immunotherapy (b)	Group I (ELA)	6.0	6	yes	yes	3.0	11.0
	LAU 444	F	27	pT3aN0M0	1.90	IV	ED	Surgery, radiotherapy, immunotherapy (a)	Group I (ELA)	22.0	20	yes	yes	13.9	30.9
	LAU 618	F	69	pT4N0M0	8.00	IIIC	ED	Surgery, radiotherapy, chemotherapy, limb perfusion, immunotherapy (a)	Group I (ELA)	12.0	12	yes		1.9	30.9
	LAU 627	M	49	pT3bN1aM0	2.23	IV	ED	Surgery	Group I (ELA)	19.0	7	yes	yes	5.0	15.0
	LAU 672	M	34	pT1aN0M0	0.70	IIIC	ED	Surgery, radiotherapy, chemotherapy, limb perfusion, immunotherapy (a)	Group I (ELA)	3.0	4	yes	yes	2.0	45.0
	LAU 701	F	70	pT3bN0M0	2.50	IIIC	NED	Surgery, limb perfusion	Group I (ELA)	8.0	8	yes		3.0	140.0
	LAU 818	M	55	pT3aN0M0	2.44	IIIB	NED	Surgery	Group I (ELA)	20.0	18	yes		8.0	61.0
	LAU 936	F	52	pT3aN0M0	2.70	IIIB	NED	Surgery, radiotherapy	Group IV (ELA+TYR)	7.0	7	yes	yes	1.0	15.0
	LAU 944	F	20	pT2N0M0	6.80	III	NED	Surgery, radiotherapy	Group I (ELA)	18.0	16	yes		24.0	137.0
	LAU 1129	M	52	pT3N0M0	2.50	IIIC	NED	Surgery, chemotherapy	Group IV (ELA+TYR)	9.0	8	yes	yes	9.0	16.0
	LAU 1144	M	68	pT3aN0M0	0.60	IV	NED	Surgery	Group IV (ELA+TYR)	9.0	8	yes	yes	9.0	29.9
	LAU 1164	M	52	pTxNxM1a	-	IV	NED	none	Group IV (ELA+TYR)	57.0	21			57.0	57.0
	LAU 1189	F	68	pT3bN2M0	4.00	IIIB	NED	Surgery	Group IV (ELA+TYR)	2.0	3			6.1	6.1
	LAU 1264	M	46	pT3bN0M0	4.00	IIIB	NED	Surgery, radiotherapy	Group IV (ELA+TYR)	44.0	26	yes		13.0	89.0
EAA	LAU 392	F	29	pT3aN0M0	2.50	III	ED	Surgery, immunotherapy (a)	Group III (EAA+TYR)	6.0	7	yes	yes	2.9	12.0
	LAU 648	M	64	pT2aN0M0	1.60	III	NED	Surgery, radiotherapy, immunotherapy (a)	Group III (EAA+TYR)	20.0	18			125.1	125.1
	LAU 660	F	22	pT2bN0M0	1.72	IV	ED	Surgery	Group II (EAA)	2.0	3	yes	yes	2.0	15.0
	LAU 706	F	64	pTxN0M0	-	III	ED	Surgery, limb perfusion, immunotherapy (c)	Group III (EAA+TYR)	4.0	4	yes	yes	3.0	44.0
	LAU 972	F	60	pT2bN1aM0	1.60	III	NED	Surgery	Group II (EAA)	23.1	20			129.0	129.0
	LAU 975	M	51	pT4aN1bM0	12.00	IIIB	NED	Surgery	Group III (EAA+TYR)	4.0	4	yes	yes	4.0	7.0
	LAU 1013	M	55	pT3bN3M0	3.00	IIIC	NED	Surgery	Group III (EAA+TYR)	8.0	8	yes	yes	7.0	24.1
	LAU 1015	M	75	pT2aN0M1a	1.20	IV	NED	Surgery	Group III (EAA+TYR)	25.0	20	yes	yes	8.0	50.0
	LAU 1017	F	28	pT3N2bM0	3.80	IIIB	ED	Surgery	Group III (EAA+TYR)	7.0	8	yes	yes	1.0	21.1
	LAU 1022	M	69	pT2bN2aM0	1.49	IIIB	NED	Surgery	Group II (EAA)	9.9	9	yes	yes	8.9	19.9
	LAU 1034	M	47	pT2aN2aM0	1.35	IIIA	NED	Surgery	Group II (EAA)	5.0	5	yes		52.0	117.0
	LAU 1090	M	68	pT3aN2bM0	3.10	IIIB	ED	Surgery	Group II (EAA)	16.1	16	yes	yes	3.0	20.0
	LAU 1106	M	36	pT2aN1aM0	1.35	IIIA	NED	Surgery	Group II (EAA)	53.0	30			107.0	107.0

† in months

* PFS: progression-free survival (in months) and OS: overall survival (in months)

ED: Evidence of disease

NED: No evidence of disease

(a) Melan-$A_{26\text{-}34\ (A27L)}$ peptide + $FluMa_{58\text{-}66}$ peptide + ASO2 (ref Lienard et al. Cancer Immunology. 2004. 4:4)

(b) Melan-$A_{26\text{-}34\ (A27L)}$ peptide + $FluMa_{58\text{-}66}$ peptide + ASO2 (ref Ayyoud et al. Cancer Res. 2003. 9:669)

(c) Melan-$A_{26\text{-}34\ (A27L)}$ peptide + p40 (ref Lienard et al. J. Immunotherapy. 2009. 32:875)

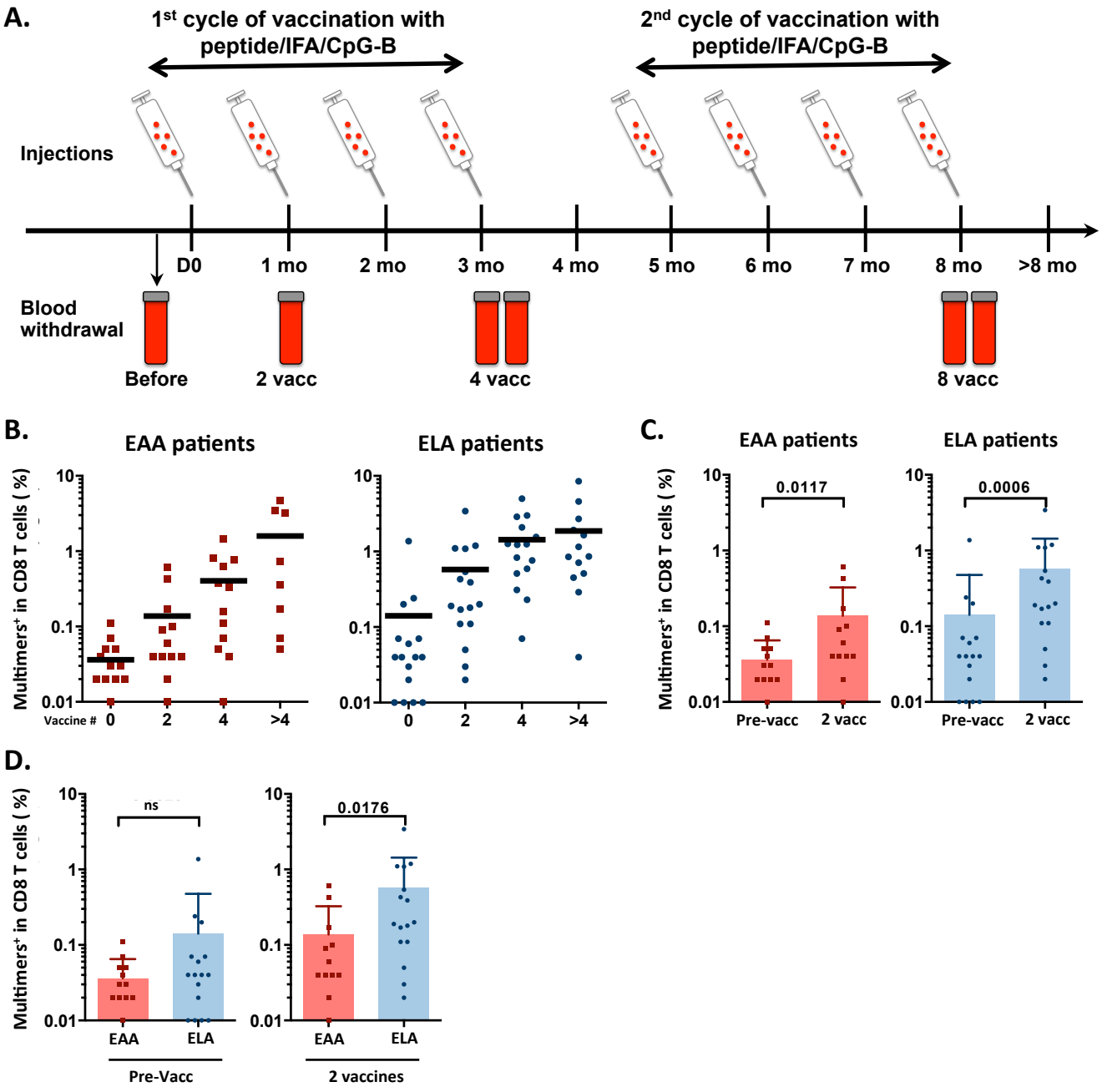
Supplementary Table 2. Patient characteristics

	All patients	Vaccine cohort native/EAA	Vaccine cohort analog/ELA	*p*-value†
Patients	29	13	16	n.s.
Female (%)	*11*	5 (39)	6 (37)	n.s.
Male (%)	*18*	8 (61)	10 (63)	
Age at start of protocol				n.s.
mean ± std.dev.	53.2 ± 16.2	53.2 ± 17.2	53.1 ± 16.2	
Range	(25 - 75)	(25 - 75)	(28 - 74)	
Vaccination duration (mo)				n.s.
mean ± std.dev.	15.6 ± 14.4	14.1 ± 14.0	16.7 ± 15.1	
Range	(2 - 57)	(2 - 53)	(2 - 57)	
# of vaccine	11.9 ± 7.5	11.7 ± 8.3	12.0 ± 7.1	n.s.
Relapse during study (%)	9 (69)	9 (69)	12 (75)	n.s.
Overall relapse (%)	10 (77)	10 (77)	14 (88)	n.s.
Mortality (%)	9 (69)	9 (69)	8 (50)	n.s.
Progression-free survival (mo)				n.s.
mean ± std.dev.	21.9 ± 36.8	34.8 ± 50.7	11.4 ± 14.2	
Range	(1.0 - 129)	(1.0 - 129)	(1.0 - 57.0)	
Overall survival (mo)				n.s.
mean ± std.dev.	51.7 ± 43.7	53.2 ± 47.7	50.6 ± 41.8	
Range	(6.1 - 140)	(7.0 - 129)	(6.1 - 140)	

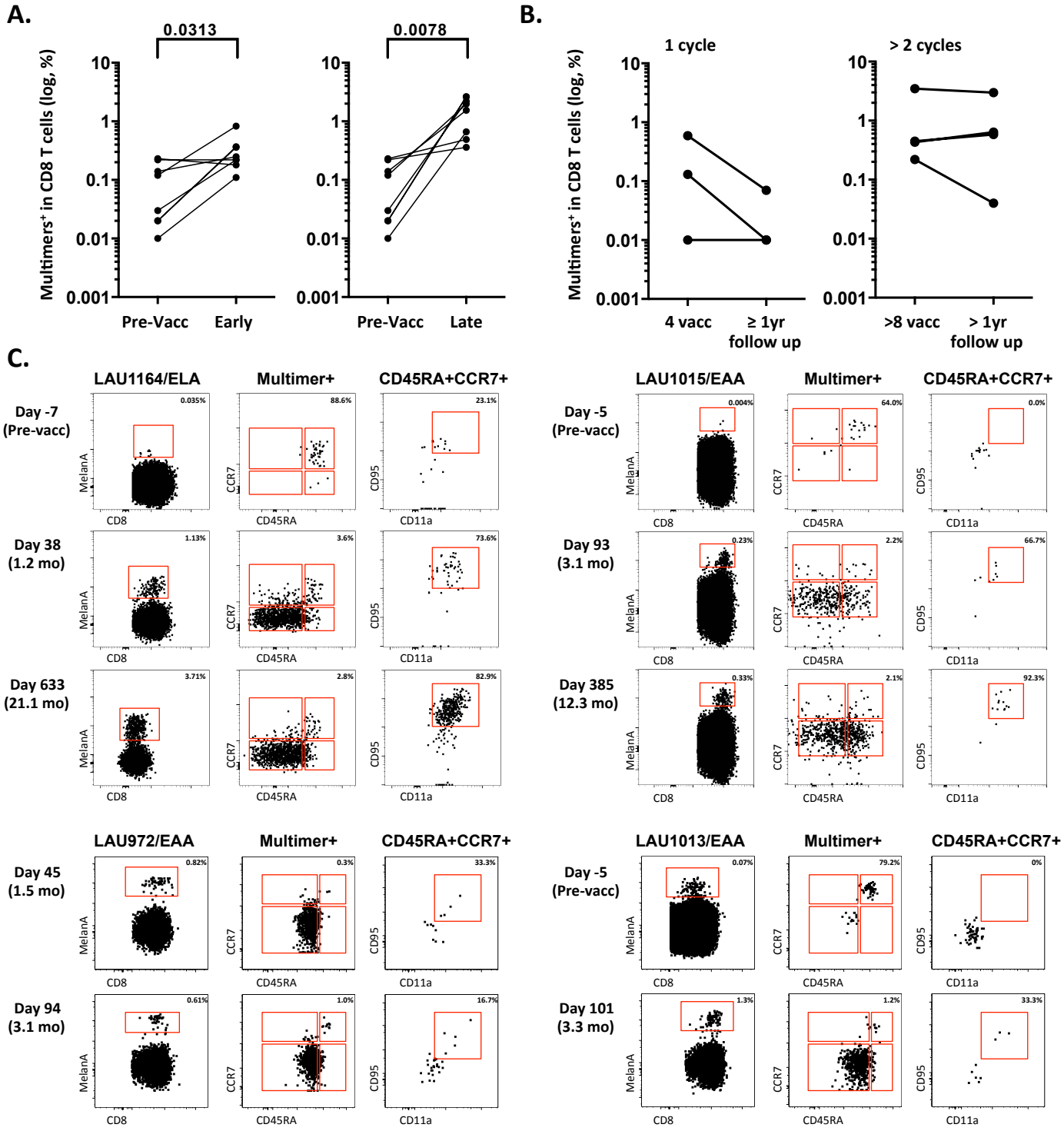
† p value by Mann-Whitney comparing the native/EAA vs the analog/ELA cohorts
n.s.: not statistically significant; mo: months

Supplementary Table 3. Adverse events

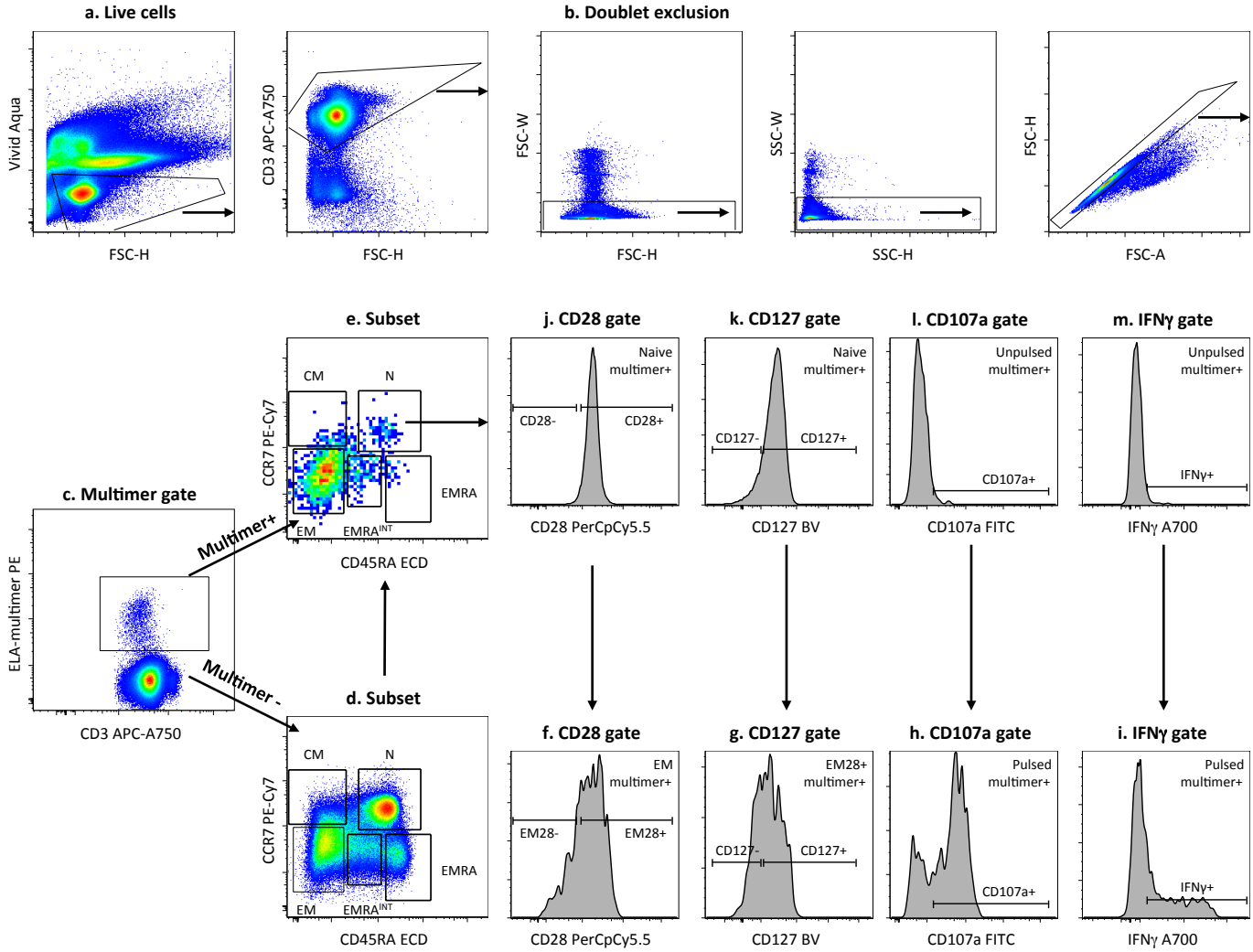
	# of events	# of patients	% of patients
Gralunoma (inflammatory)	28	8	27.6%
Fatigue	13	3	10.3%
Gralunoma (residual)	12	3	10.3%
Myalgia	12	5	17.2%
Head Ache	10	3	10.3%
Arthralgia	9	3	10.3%
Nausea	4	2	6.9%
Pain	4	4	13.8%
Anti-nuclear antibodies	3	2	6.9%
Chills	3	1	3.4%
Common Cold	3	2	6.9%
Diminution Karnofsky	3	1	3.4%
Erythma	3	2	6.9%
Malaise	3	2	6.9%
Pain (joint)	2	2	6.9%
Anxiety	1	1	3.4%
Bronchitis	1	1	3.4%
Diarrhea	1	1	3.4%
Fever	1	1	3.4%
Gastroenteritis	1	1	3.4%
Gout	1	1	3.4%
Lost of appetite	1	1	3.4%
Pain (muscular)	1	1	3.4%
Pressure	1	1	3.4%
Sweating	1	1	3.4%
Weight Gain	1	1	3.4%



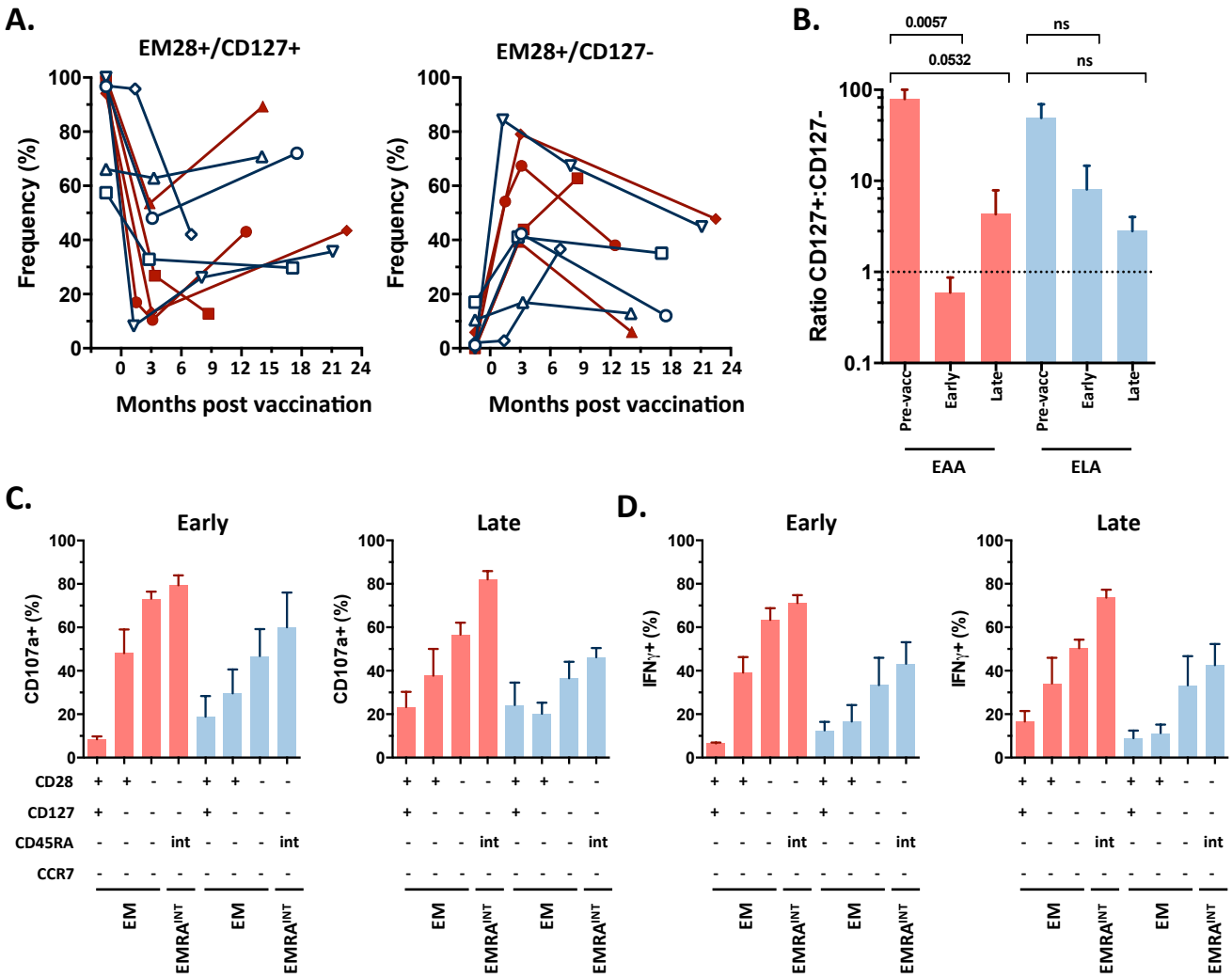
Supplementary Figure 1*: Ex vivo* frequencies of circulating Melan-A-specific CD8 T cell following peptide vaccination. (A) Blood samples of melanoma patients receiving monthly Melan-A peptide, IFA and CpG vaccinations were harvested before vaccination (0 or pre-vacc) and at regular time points following vaccination. Melan-A-specific CD8 T cell frequencies were quantified *ex vivo* by multimer staining following CD8 enrichment. **(B)** Tumor-specific T cells for native/EAA (red, n = 13) and analog/ ELA (blue, n = 16) vaccinated patients according to the number of vaccines. **(C)** Comparisons of Melan-A-specific CD8 T cell frequencies at pre-vaccination and after two (2) vaccine injections in native/EAA (left panel) or analog/ELA (right panel) vaccinated patients. *p*-values by Wilcoxon matched-pairs signed rank test. **(D)** Comparison of the Melan-A-specific T cell frequencies at pre-vaccination and after two vaccine injections between native/EAA- and analog/ELA-vaccinated patients (red and blue bars, respectively). *p*-values are by Mann-Whitney U-test.



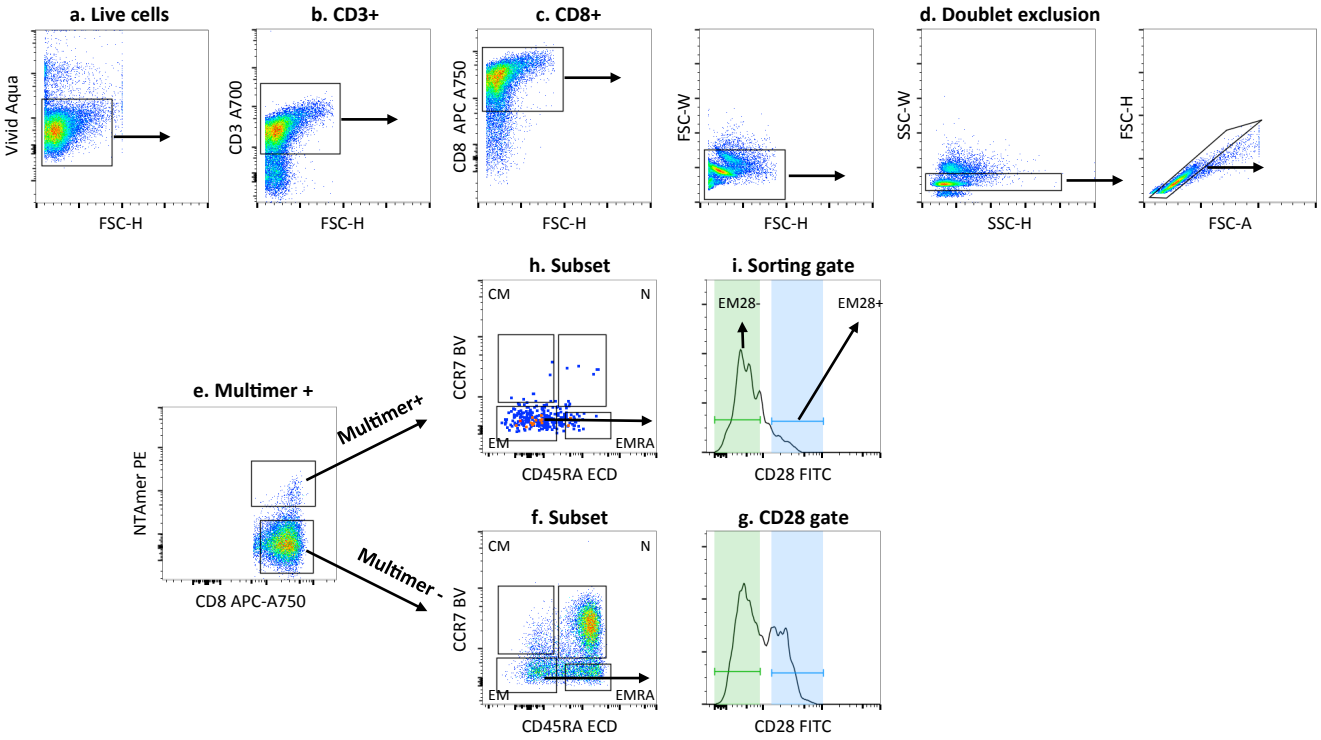
Supplementary Figure 2: *Ex vivo* characterization of tumor-specific CD8 stem cell-like memory T cells before and after peptide vaccination. (A) Comparisons of the frequencies of circulating Melan-A-specific CD8 T cells before vaccination (pre-vacc) and at early (between 2 to 4 vaccines; left panel) or late (>4 vaccines and >6 months; right panel) time-points after vaccination. *p* values by Wilcoxon matched-pairs signed rank test. **(B**) Impact of the number of vaccination cycles on the stability of tumor-specific T cell frequencies over time. Three patients who received only one cycle (4 vaccines) and four patients receiving > 2 cycles (> 8 vaccines) were immune monitored after more than 1 year during which they received no further vaccination. **(C)** Gating strategy for the analysis of tumor-specific CD8 T_{SCM} cells (CD8+/multimer+, CD45RA+/CCR7+, CD95+/CD11a+) for one analog/ELA and three native/ EAA peptide vaccinated patients. Mo; months post vaccination.



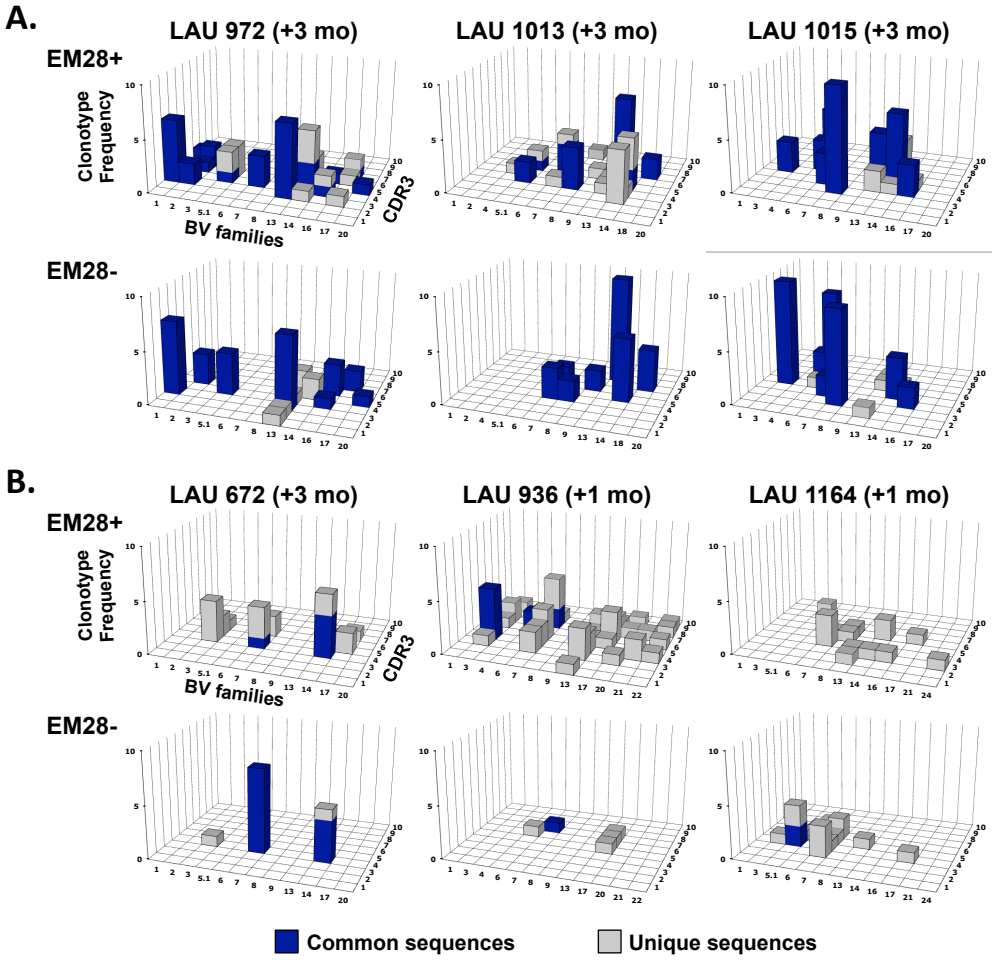
Supplementary Figure 3: Gating strategy for functional analyses following recognition of T2 target cells pulsed with native/EAA peptide. Following dead cell **(a)** and doublet exclusions **(b)**, we gated on multimer+ and multimer- **(c)** live enriched CD8+CD3+ T cells. On the multimer- gate, the various subsets gates were established **(d)**, which included naïve (CD45RA+/CCR7+), central memory (CM, CD45RA-/CCR7+), effector memory (EM, CD45RA-/CCR7-), effector memory CD45RA^{INT} (EMRA^{INT}, CD45RA^{INT}/CCR7-) and effector memory CD45RA+ (EMRA, CD45RA+/CCR7-). Subsets gates were then applied to the multimer+ CD3+ T cells **(e)**. Using histogram plots, positive and negative gates were established using pre-determined control subsets for the following markers: CD28 **(f),** CD127 **(g)**, CD107a **(h)** and IFNγ **(i)**. These gates were then applied to the relevant subsets **(j, k, l** and **m)**. For combinatorial gating, the histogram gates were added sequentially. For example, to quantify the frequency of CD107a+ T cells within the EM28+/CD127-, the gating strategy was multimer+ -> EM gate (CD45RA-/CCR7-) -> CD28+ gate -> CD127- gate -> CD107a+ gate. All gates were adapted for every patient and time point.



Supplementary Figure 4: Impact of peptide vaccination on tumor antigen-specific CD8 T cell differentiation. **(A)** Kinetics of the differentiation of effector-memory (EM, CD45RA-/CCR7-) multimer+ CD8+ T cells in terms of the expression of CD127 (IL-7Rα). Frequencies of EM28+/CD127+ or EM28+/CD127- within total EM28+ T cell subset. Native/EAA patients (n = 4, red lines) and analog/ELA patients (n = 5, blue lines). (**B)** Quantification of the ratio of CD127 expression within the EM28+ subset in terms of pre-vaccine (prior to vaccination), early (after 2 to 4 vaccine and <3 months after the start of vaccination) and late time-points (>4 vaccines and >3 months after the start of vaccination). *p* values by Kruskal-Wallis. **(C-D)** Increased acquisition of surface CD107a **(C)** and intracellular IFNγ **(D)** expression with regards to the progressive differentiation from EM28+/CD127+, EM28+/CD127-, EM28- and EMRA^{INT}. Native/EAA patients (n = 4, red bars) and analog/ELA patients (n = 5, blue bars). Means with standard errors are depicted.



Supplementary Figure 5: Gating strategy for single cell sorting of tumor-specific CD8 T cells following early and late native/EAA or analog/ELA peptide vaccination time-points. Following gating on live **(a)**, CD3+ **(b)**, CD8+ **(c)** T cells and doublet exclusions **(d)**, we gated on multimer+ and multimer- **(e)** CD8+CD3+ T cells. On the multimer- gate, the various subsets gates were established **(f)**, which included naïve (CD45RA+/CCR7+), central memory (CM, CD45RA-/CCR7+), effector memory (EM, CD45RA-/CCR7-), and effector memory CD45RA+ (EMRA, CD45RA+/CCR7-). Subsets gates were then applied to the multimer+ CD8+ T cells **(h)**. Using histogram plots, CD28positive and CD28negative gates were established using pre-determined control subsets for the CD28 marker **(g)**. These gates were then applied to the effector-memory (EM) subset **(i)**.



Supplementary Figure 6: *Ex vivo* TRBV repertoire diversity and clonotype frequency of Melan-A-specific CD8 T cell subsets at early time-points after peptide vaccination. **(A, B**) TRBV spectratyping and sequencing was performed on cDNA obtained from individually sorted 5-cell samples of $EM28^{pos}$ (upper panel) and $EM28^{neg}$ (lower panel) T cells isolated from blood samples at early time-points (< 3 months) after native/EAA **(A)** or analog/ELA **(B)** peptide vaccination. Dominant TCR clonotypes are defined by identical BV-CDR3-BC sequences found at least twice, and non-dominant single TCR clonotypes by their unique TCR BV/CDR3 size length and/or BV-CDR3-BC sequence. Data are represented as total number (y-axis) of dominant (filled area) and single (grey area) clonotypes of defined TRBV family (x-axis) versus defined CDR3 size length (z-axis).